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(71) Applicant (*for all designated States except US*): THERMO FAST UK LIMITED [GB/GB]; Saxon Way, Bar Hill, Cambridge CB3 8SL (GB).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): LYNDIN, Nikolai Mikhailovich [RU/RU]; Nagornaya Street 4-20, Moscow, 113186 (RU). SYCHUGOV, Vladimir Alexandrovich [RU/RU]; Simonovsky val 10-84, Moscow, 113186 (RU). TISHCHENKO, Alexander Valentinovich [RU/RU]; Lomonosovsky Prospect 23-356, Moscow, 117311 (RU). USIEVICH, Boris Alexandrovich [RU/RU]; Miklukho-Maklaya Street 55-305, Moscow, 117279 (RU).

(74) Agent: JONES, Stephen, Anthony; Lewis & Taylor, 144 New Walk, Leicester LE1 7JA (GB).

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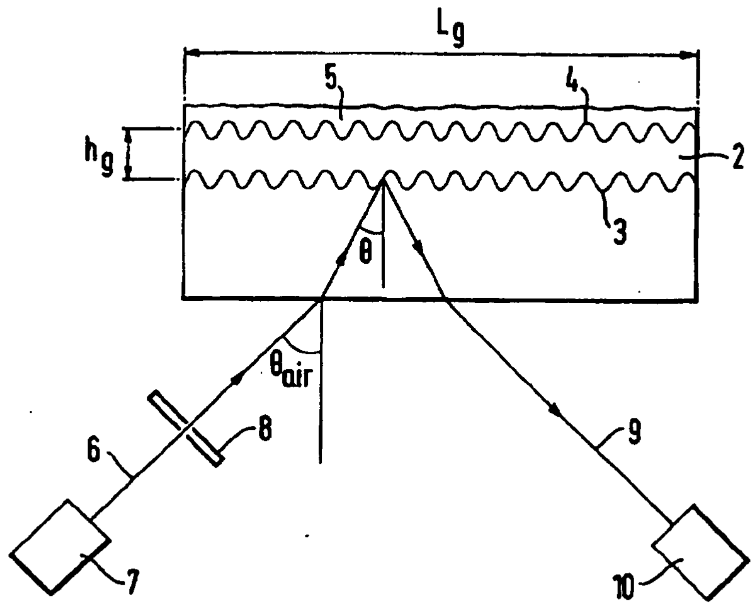

(57) Abstract

A method for monitoring the interaction of molecular species utilizes a sensor device comprising a substrate (1) with a waveguide (2) formed on the surface thereof. A grating (3) is formed in one of the surfaces of the waveguide (2). A beam of light (6) is incident on the grating (3) and the angle of incidence at which maximum reflection occurs is monitored. A first molecular species is immobilized on the waveguide (2). Changes in the angular position of the reflection maximum provide an indication of interaction of the first molecular species with a second molecular species contained in a sample brought into contact with the sensor device.

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Title - Analytical Methods and Apparatus

This invention relates to methods and apparatus for the analysis of analyte molecular species in a sample.

Devices are known with a surface on which is immobilized a layer of biomolecules having an affinity for other molecules ("the analyte") in a sample under test. Such devices are commonly referred to as biosensors. The immobilized biomolecules and the analyte may, for example, constitute a specific binding pair such as an antigen-antibody pair. Interaction of the two members of the pair causes a change in the physical properties of the device. This change can be used as an indicator of the presence and/or concentration of the analyte, the strength and/or progress of the interaction etc.

In many biosensors, it is the optical properties of the device which are monitored. One class of optical biosensor comprises a waveguide in the form of a thin layer of relatively high refractive index material coated on a substrate of optically transparent lower refractive index material. Biomolecules are immobilized on the surface of the waveguide and the interface between the substrate and the waveguide is irradiated with a beam of light.

Means are generally provided to facilitate coupling of light into the waveguide. The optical properties of the device will depend on the nature of those means, as well as on other factors including the wavelength of the incident light, the materials used for the waveguide and the substrate, the thickness of the waveguide etc. In general, however incident light is coupled to a greater or lesser extent into the waveguide. Chemical binding events at or in the vicinity of the waveguide surface will cause a localized change in refractive index, which in turn causes a change in the coupling characteristics of the device. This provides a means for monitoring interactions between the immobilized biomolecules and the analyte molecules.

One form of coupling means which has been proposed is a grating structure formed, for instance, in the interface between the substrate and the waveguide. In general, light incident will be reflected, transmitted or scattered into the various diffraction orders of the grating. Further, at

certain angles of incidence, where a diffraction order matches the waveguide propagation condition, light will be coupled into the waveguide.

Here, light will propagate in the guide parallel to the substrate surface, where it will continue to interact with the grating. The light will couple back out of the waveguide via the various diffraction orders and into free-space beams. This outcoupled light will include beams in the same direction as the transmitted and reflected, uncoupled beams.

Attempts to measure the coupling condition are hampered by overlap of the waveguide derived beams and the uncoupled, transmitted or reflected components. This leads to measurements of low contrast.

One approach to this problem is to provide a pair of grating structures separated by an unmodulated region. Light incident on one of the gratings is coupled into the waveguide and is then coupled out by the second grating. The coupled-out light is thus spatially separated from the light reflected or transmitted at the first grating. However, the need for the provision of two gratings is a disadvantage.

In another approach, a single grating structure is employed, the grating structure being a superposition of grating elements having two different periodicities. Light incident on the grating structure at a first angle is coupled into the waveguide by the grating element with a first periodicity. It is then coupled out by the grating element having a second periodicity, at a different angle. The coupled-out light is thus angularly separated from the reflected light. Such a bidiffractive grating is relatively difficult to fabricate.

There have now been devised methods for monitoring the interaction of molecular species, and devices suitable for use in such methods in which light is coupled into a waveguide by a grating structure, which overcome or substantially mitigate the above-mentioned disadvantages.

According to a first aspect of the invention, a method for monitoring the interaction of a first molecular species in a sample with a second molecular species comprises

providing a sensor device comprising a substrate having a waveguide formed on at least part of the surface thereof, the waveguide having a first major surface which constitutes an interface between the waveguide and the substrate and a second major surface upon which the second molecular species is immobilized, at least a region of the first and/or second major surface being formed with a periodic refractive index modulation;

contacting a sample containing the first molecular species with the second major surface;

irradiating said periodic refractive index modulation with a beam of incident monochromatic light,

varying the angle of incidence of said light or the wavelength of said light through a range of angles or wavelengths respectively, said range including an angle or a wavelength, as the case may be, at which a guided mode is excited in said waveguide;

monitoring the intensity of light reflected from said periodic refractive index modulation as a function of the angle of incidence or wavelength of the incident light; and

determining the angle of incidence or wavelength at which the intensity of the light reflected from the periodic refractive index modulation is a maximum.

In general, the incident light will be partially reflected from the periodic refractive index modulation, partially transmitted and partially coupled into the waveguide. The method according to the invention is advantageous primarily in that, at a certain angle of incidence, a near-total reflection of the incident light beam can be achieved. The incident light excites a guided mode in the waveguide. This guided mode propagates a certain distance and is then coupled out, back into the substrate and into the superstrate adjacent the waveguide. It is possible by appropriate choice of parameters to achieve almost complete destructive interference of the transmitted components at the coupling angle. This interference is between the zeroth order transmitted beam and the beam radiated from the out-coupled guided wave into the superstrate, ie the material beyond the waveguide (in the direction of the incident light beam).

In such a case there is a correspondingly high intensity of the reflected light at the propagation angle, and this is relatively easily monitored. Such high reflection may be termed "anomalous" or "abnormal" reflection. Interaction of the molecular species immobilized on the waveguide surface with analyte molecules in a sample which is contacted with the waveguide causes a local change in refractive index in the vicinity of the waveguide surface. This in turn changes the angle of incidence or wavelength at which the reflection maximum occurs, providing a sensitive indicator of the chemical interaction taking place at the surface.

The method according to the invention utilises a sensor device with only a single periodic refractive index modulation, and may therefore be easier and/or less expensive to fabricate than devices incorporating multiple gratings or bidiffractive gratings.

By the words "reflected" and "reflection" as used herein we mean the return of the incident light beam from the waveguide through the substrate at an angle equal and opposite to the angle of incidence. Although this is superficially similar to conventional specular reflection, the mechanism of "reflection" in the present case includes diffraction effects from the grating.

The periodic refractive index modulation is preferably a surface relief profile or a grating formed in the surface of the substrate to which the waveguide coating is applied and/or in the surface of the waveguide on which the second molecular species is immobilized. Where the waveguide is formed by a deposition process, the relief profile is preferably formed on the substrate and the waveguide then deposited, e.g. by a chemical vapour deposition process. The periodic refractive index modulation may be formed in one or both major surfaces of the waveguide. Modulation of both surfaces may be created by design or may be a consequence of the fabrication process used. For example, deposition of the waveguide on a relief profile formed in the substrate may result in the surface of the waveguide having a similar corrugation to that of the substrate.

The corrugation may have a variety of forms. For example, it may be sinusoidal, rectangular, triangular (saw-tooth) or trapezoidal.

The substrate is conveniently a chip, eg of glass or silica, and, in use, the superstrate is most

commonly an aqueous sample. The waveguide is preferably of relatively high refractive index, e.g. a material having a refractive index of, say, 1.80 to 2.50. Suitable materials for the waveguide include hafnium dioxide, silicon nitride, tantalum pentoxide and titanium oxide.

The optimal physical dimensions of the sensor device, grating etc will depend on the wavelength of the incident light. In the following description, the values given for the waveguide thickness, grating depth and period, light beam diameter etc encompass those suitable for commonly-used wavelengths, eg a wavelength of 633nm.

Typically, the waveguide may have a thickness of the order of 50nm to 300nm, more preferably 100nm to 200nm. We particularly prefer the thickness of the waveguide to be in the range 140nm to 180nm.

The depth of the periodic refractive index modulations (e.g. the corrugations in the surface of the substrate) is preferably less than 50nm, more preferably less than 25nm, eg typically 2nm to 20nm or 5nm to 10nm. The period of the grating is typically 300nm to 1000nm, more preferably 600nm to 1000nm.

In general, near total reflection of the incident light from the waveguide can be achieved when the radius of the incident beam at the grating exceeds the coupling length of the guided mode in the waveguide, and the dissipative and scattering losses in the waveguide are far less than the radiative losses. As explained above, this "anomalous" or "abnormal" reflection occurs when there is destructive interference of the transmitted light at the angle of propagation within the waveguide.

Thus, the invention provides a method of monitoring the interaction of the first and second molecular species, which method utilizes a device of the type generally described above and comprises monitoring the angle of incidence (or wavelength) at which the abnormal reflection maximum condition occurs.

Although, in theory, the reflection of the incident beam may be nearly total, in practice

instrumental and other factors usually mean that the intensity of the reflected beam is somewhat less than this. Nonetheless, the reflected intensity is still sufficiently great to provide a clear maximum.

In general, satisfactory results can be obtained when the maximum reflected intensity (eg occurring when the anomalous reflection conditions are met) is greater than about 20% of the intensity of the beam incident upon the substrate, more preferably greater than 30% or 40%.

The position of the reflection maximum may be determined only before and after the first and second molecular species are brought into contact, eg to provide a qualitative and/or quantitative indication of the presence of the first molecular species in the sample. Alternatively, the position of the reflection maximum may be monitored periodically or continuously in real time, eg to provide information on the progress and kinetics of the interaction.

Where the device is irradiated from below the substrate, it is strongly preferred that reflection from the underside of the substrate be minimal. To achieve this, the beam of incident light is preferably TM-polarized and the angle of incidence of the light beam on the substrate is preferably close to the Brewster angle, ie the angle $\theta_B = \tan^{-1}(n_S)$ where n_S is the refractive index of the substrate. Alternatively, the underside of the substrate may carry an anti-reflection coating.

The beam of incident light preferably has a diameter of less than 10mm, eg 1mm to 8mm, more preferably less than 6mm, eg 2mm to 5mm.

It is also preferred that the point at which the beam of incident light is incident upon the periodic refractive index modulation be offset from the centre of that modulation. The length of the grating is preferably less than 20mm, more preferably less than 10mm, eg about 7mm. In such a case, the incident light beam is preferably offset from the centre of the grating by less than 1mm, eg 0.2 to 0.6mm. The effect of this offset is to minimise the grating area required to achieve a given sensitivity. Since a major contributor to the cost of fabrication is the size of the grating used, any measure which reduces the size of grating required (without reducing the

device sensitivity) is beneficial.

As mentioned above, the optimal dimensions for the grating etc are wavelength-dependent. Relating the various parameters to the wavelength λ, the following ranges are typical:

waveguide thickness: $\lambda/12$ to $\lambda/2$

grating depth: $\lambda/300$ to $\lambda/12$, more preferably $\lambda/300$ to $\lambda/30$

grating period: $\lambda/2$ to 2λ, more preferably λ to 1.5λ.

The background reflection, ie the intensity of the reflected light at angles/wavelengths away from that at which a guided mode is coupled into the waveguide, may be reduced by choosing a waveguide thickness which minimises Fresnel reflection.

The means for generating the incident beam of monochromatic light may be any conventional source of monochromatic radiation, most preferably a laser light source. By "light" is meant in this context not only visible light, but also wavelengths above and below that range, e.g. in the infra-red and ultra-violet.

Appropriate collimating and/or polarising optical components may be interposed between the light source and the sensor, as required.

The beam of incident light may be scanned sequentially through a range of angles of incidence. This may be achieved by optical manipulation of the light beam, or by physical movement of the light source.

Where the wavelength of the incident light is varied, this may be achieved by the use of a tunable light source such as a tunable laser.

The means for monitoring the intensity of the radiation reflected from the device may be a

conventional detector , e.g. a photoelectric detector such as a charge coupled device (CCD) or an array of such devices. It may be necessary for the detector to be moved in synchronism with variation in the angle of incidence.

The second molecular species is immobilised on the surface of the waveguide. Suitable methods for such immobilization will be familiar to those skilled in the art. The second molecular species may be immobilized directly on the waveguide surface, or may be indirectly linked to the surface. The second molecular species may be linked to the surface through covalent bonds to intermediate chemical species or by physical interaction with other immobilised species, or may be chemically or physically bound to a coating layer, eg a hydrogel matrix or other polymeric coating, applied to the waveguide surface.

It may be possible to use the apparatus and method of the invention in a manner which provides internal compensation for systematic errors such as temperature fluctuations or variations in the position of the incident beam etc. To this end, a dual-mode waveguide may be used or simultaneous irradiation at two different wavelengths, giving rise to a pair of resonances (ie a pair of angles/wavelengths at which coupling occurs). The sensitivities of the two resonances (ie the dependence of the positions of the resonances on the interaction of first and second molecular species) will generally be different.

According to a second aspect of the present invention, apparatus for monitoring the interaction of a first molecular species with a second molecular species comprises

a sensor device comprising a substrate having a waveguide formed on at least part of the surface thereof, the waveguide having a first major surface which constitutes an interface between the waveguide and the substrate and a second major surface upon which the second molecular species is immobilized, at least a region of the first and/or second major surface being formed with a periodic refractive index modulation;

means for irradiating said periodic refractive index modulation with a beam of incident monochromatic light,

means for varying the angle of incidence of said light or the wavelength of said light through a range of angles or wavelengths respectively, said range including an angle or a wavelength, as the case may be, at which a guided mode is excited in said waveguide: and

means for monitoring the intensity of light reflected from said periodic refractive index modulation as a function of the angle of incidence or wavelength of the incident light;

means for determining the angle of incidence or wavelength at which the intensity of the reflected light is a maximum.

Preferably, the means for irradiating the periodic refractive index modulation includes means for irradiating the substrate at an angle or range of angles of incidence which is close to, or includes, the Brewster angle.

Preferably, the apparatus further comprises means for polarizing the incident light beam such that the light incident upon the sensor device is TM-polarized.

Preferably, the dimensions and form of the waveguide and the grating are as described above.

The invention will now be described in greater detail, by way of illustration only, with reference to the accompanying Figures, in which

Figure 1 is a schematic view (not to scale) of a biosensor device according to the invention;

Figure 2 shows the mode sensitivity S_0, as a function of waveguide thickness, for a waveguide formed on a glass (broken lines) or silica (solid lines) substrate;

Figure 3 shows the Fresnel reflection coefficient R_F as a function of waveguide thickness, for a waveguide formed on a glass (broken lines) or silica (solid lines) substrate;

Figure 4 represents the power of radiation reflected from the biosensor device of Figure 1 as a

function of the deviation of the angle of incidence from an angle at which the reflection is a maximum;

Figure 5 shows the radiative loss coefficient α as a function of grating groove depth for a biosensor device similar to that shown in Figure 1 (with a rectangular grating profile); and

Figure 6 illustrates stages in the manufacture of a sensor device as shown in Figure 1.

Referring first to Figure 1, a biosensor device comprises a substrate in the form of a chip 1 (eg of glass or silica) approximately 7mm square and 2mm in thickness. The chip 1 has a refractive index of 1.46. Coated on the upper surface of the chip 1 is a waveguide 2.

The interface between the chip 1 and the waveguide 2 is formed with a periodic relief profile or grating 3 (the grating 3 is shown as being sinusoidal though in practice a generally rectangular profile is produced by the method of fabrication described below). The waveguide 2 is formed by deposition on the chip 1 and a corresponding relief profile 4 may thus be formed also on the upper surface of the waveguide 2. A layer 5 of biomolecules, eg antibodies, is immobilized on the upper surface of the waveguide 2 in a known manner.

A beam 6 of monochromatic light (λ= 633nm) is produced by a laser light source 7. The beam 6 passes through a polarizer 8 and is incident on the underside of the chip 1 at an angle θ_{air}. The angle of incidence θ_{air} of the light beam 6 on the base of the chip 1 may be varied through a range of angles. Such variation may be brought about by mechanical movement of the light source 7 and/or optical deviation of the incident beam 6. The intensity of a reflected beam 9 of light reflected from the device is measured by a suitable detector 10.

The process by which the structure and operating parameters of the device of Figure 1 are optimised will now be described. The process has three stages:

a) Selection of angle of incidence θ_{air}

Fresnel reflection from the underside of the chip 1 is minimized by arranging the polarizer 8 such that the incident light beam 6 is TM-polarized. Advantage is then taken of the Brewster effect by choosing the angle of incidence θ_{air} according to

$$\theta_{air} = \tan^{-1} n_S$$

where n_S = refractive index of the chip 1.

This gives an angle of incidence θ on the grating 3 which is

$$\theta = \sin^{-1}\left[\frac{\sin \theta_{air}}{n_S}\right]$$

For a glass chip 1, with n_S=1.51, θ_{air}=56.49° and θ=33.51°. If silica were used as the substrate material, the corresponding figures would be n_S=1.46, θ_{air}=55.59° and θ=34.41°.

b) Determination of the waveguide parameters

The optimum waveguide thickness h_g for a given waveguide refractive index is now determined. First, the waveguide thickness is determined by maximizing the TM_0 mode sensitivity S_0. An example of the dependence of the sensor sensitivity on the waveguide thickness, and of the optimum value h_{gS} corresponding to the maximum sensitivity is shown in Figure 2. This shows the sensitivity S_0 as a function of waveguide thickness h_g for a silica substrate (solid curves) and a glass substrate (broken curves) at each of three values of waveguide refractive index n_g (2.0, 2.1 and 2.2). As can be seen, the waveguide thickness h_g which gives greatest sensitivity (which we designate h_{gS}) varies between about 130nm and 150nm.

Secondly, since the waveguide 2 has two boundaries, it is possible to use the effect of interference of reflections from these two interfaces to substantially reduce the overall Fresnel reflection for an incidence angle close to but outside the resonance.

Typical data for Fresnel reflection coefficient R_F versus waveguide thickness (for a planar waveguide formed on a silica or glass substrate - solid and broken lines respectively) is presented in Figure 3. In the case of a silica substrate, data is shown for two values of waveguide refractive index n_g=2.0 and 2.1. The waveguide thickness corresponding to the minimum reflection is denoted h_{gR}. The optimum waveguide thickness is a compromise between the condition of maximum sensitivity $h_g=h_{gS}$, and condition $h_g= h_{gR}$ for minimum Fresnel reflection. Fortunately, these two conditions are close to each other. The waveguide thickness h_g is therefore chosen in the interval between h_{gS} and h_{gR}. Preferably, h_g is chosen closer to h_{gR} to get a high quality reflection peak while losing no more than 10% sensitivity. For example, n_g=2.0 leads to an optimum waveguide layer thickness h_g=0.17µm; n_g=2.1 leads to an optimum waveguide layer thickness h_g=0.165µm. The reflection coefficient R_F is ~0.1% in both cases.

c) Determination of the grating parameters

Now, for a given waveguide structure, it is possible to calculate the effective refractive index n_{e0} of the fundamental mode and then the desired period Λ of the waveguide corrugation:

$$\Lambda = \frac{\lambda}{n_{e0} - n_S \sin\theta}.$$

In order to obtain high reflection it is necessary that the following condition be satisfied:

$$\frac{\alpha w}{\cos\theta} \gg 1$$

where w is the radius of the incident Gaussian beam 6 (ie half the full width of the beam at half-height), and α is the total loss coefficient in the waveguide. Figure 4 shows an example of the dependence of the normalized reflected power R on the angle of incidence (shown as a deviation from the angle giving maximum reflection). The peak quality may be expressed in terms of a parameter $F_{opt} = R_{max}/\Delta\theta$ where R_{max} is the amplitude of the peak and $\Delta\theta$ is its angular width at half-height. A "figure of merit" may also be obtained by multiplying F_{opt} by the mode sensitivity

S_0.

It is found that for a grating of length L_g the total losses α, beam radius w, and beam centre position Δz on the grating area should satisfy the following approximate conditions:

$$\alpha \approx \frac{4.06}{L_g} \qquad \frac{w}{\cos\theta} \approx 0.44L_g \qquad \Delta z \approx 0.44L_g$$

For a grating length L_g = 7mm, the following figures are obtained:

Full loss coefficient	$\alpha=5.8\text{cm}^{-1}$
Radius of Gaussian beam in the grating plane	$\frac{w}{\cos\theta} = 3.1\text{mm}$
Radius of Gaussian beam in the substrate	w=2.5mm
Distance of beam centre to grating edge	Δz=3.1mm

The value of the loss coefficient in the waveguide determines the necessary groove depth. Considering, as an approximation, a sinusoidal groove profile and a single corrugated boundary, the groove depth can be calculated using known analytical formulae [(see, for example, Y. Yamamoto et al, IEEE J. Quant. Electron., QE-14, 620-625 (1978)].

Figure 5 shows the radiative loss coefficient α as a function of groove depth σ for a silica substrate (solid lines) at two values of waveguide refractive index n_g = 2.0 and 2.1, and for a glass substrate (broken line) at n_g = 2.1. In each case, the groove depth σ corresponding to the previously determined value of α = 5.8cm^{-1} can be read off.

In summary, for a given choice of substrate and waveguide materials, and a given wavelength, the optimum angle of incidence is chosen to minimise reflection from the underside of the

substrate, the waveguide thickness is chosen as a compromise between maximum sensitivity and minimum Fresnel reflection, and finally the grating parameters (period, groove depth) are optimised.

Table I summarizes the optimum parameters derived as described above for three different combinations of waveguide and substrate materials, the wavelength of the light used being 633nm in all cases and the grating length 7mm.

TABLE 1

Parameter	Structure 1	Structure 2	Structure 3
Waveguide parameters			
Substrate refractive index	1.46	1.46	1.51
Waveguide refractive index	2.0	2.1	2.1
Waveguide thickness h_g (nm)	160	165	164
Effective refractive index (without grating)	1.61308	1.68258	1.69759
Grating parameters			
Grating period Λ (µm)	0.8	0.74	0.73
Groove depth σ (nm)	8.6	6.8	7.2
Loss coefficient α (cm^{-1})	5.80	5.69	6.02
Effective refractive index (with grating)	1.61337	1.68291	1.69789
Geometrical parameters			
Incidence angle θ_{air} in the air (degrees)	55.32	55.87	56.21
Incidence angle θ in the substrate (degrees)	34.28	34.54	33.39
Incident beam radius w_{air} in the air (mm)	1.77	1.77	1.66
Incidence beam radius w in the substrate (mm)	2.56	2.61	2.5
Beam centre position Δz at the grating (mm)	3.10	3.16	2.99
Biosensor merit			
Mode sensitivity S_0 (μm^{-1})	0.279	0.304	0.281
Peak width $\Delta\theta$ (degrees)	0.0084	0.0083	0.0082
Maximum reflection R_{max} (%)	51.7	53.5	54.2
Reflection out of resonance R_F	0.4	0.05	0.09
Reflection from the substrate (%)	0.0007	0.0008	0.0009
Peak quality $F_{opt} = \frac{R_{max}}{\Delta\theta}$ (%/degrees)	6155	6446	6610
Figure of merit S_0F_{opt} (%/degrees/µm)	1717	1960	1717

It can be seen from Figure 4 that there is one particular angle at which greatly increased reflection occurs with very low background. This angle is sensitive inter alia to the refractive index in the immediate vicinity of the upper surface of the waveguide 2. Changes in this refractive index, e.g. as a result of binding of biomolecules in a sample contacted with the surface of the waveguide 2 with the immobilized biomolecules on that surface. cause shifts in the angular position of the reflection maximum. Such shifts indicate the extent of interaction between the analyte biomolecules in the sample and the immobilized biomolecules.

Thus, the sensor device according to the invention may be used to investigate such interactions. For example, the angle of incidence θ of the light beam 6 may be scanned through a range of angles encompassing the angle at which the reflection maximum occurs and the position of that maximum is determined. The sample to be investigated is then contacted with the surface of the waveguide 2 and the process repeated. Continuous real-time measurements may be made to follow the interaction process.

Most conveniently, the measured data (reflected intensity as a function of angle of incidence and time) are digitised and stored in a computer unit connected to the sensor device.

It can be seen from Table I that the optimum groove depth is very small. The fabrication of such a grating structure presents certain difficulties but one method by which it can be achieved is as follows (see Figure 6).

First, a layer of photoresist 61 is deposited onto the surface of the substrate 1 (Figure 6a). The photoresist has a thickness of approximately 700nm. The grating pattern is then exposed into the photoresist 61, eg through a mash or by a direct holographic method using crossed laser beams, and developed to reveal the grating pattern (Figure 6b). The surface of the substrate 1 is then etched through the photoresist, eg using reactive ion-etching, ion-beam etching or wet etching, the exposed surface regions of the substrate 1 being removed (see Figure 6c) to the desired depth (typically 5-10nm). The remaining photoresist is then removed (Figure 6d) and the waveguide 2 then deposited (Figure 6e), eg by a chemical vapour deposition process.

Claims

1. A method for monitoring the interaction of a first molecular species in a sample with a second molecular species comprises

providing a sensor device comprising a substrate having a waveguide formed on at least part of the surface thereof, the waveguide having a first major surface which constitutes an interface between the waveguide and the substrate and a second major surface upon which the second molecular species is immobilized, at least a region of the first and/or second major surface being formed with a periodic refractive index modulation;

contacting a sample containing the first molecular species with the second major surface;

irradiating said periodic refractive index modulation with a beam of incident monochromatic light,

varying the angle of incidence of said light or the wavelength of said light through a range of angles or wavelengths respectively, said range including an angle or a wavelength, as the case may be, at which a guided mode is excited in said waveguide;

monitoring the intensity of light reflected from said periodic refractive index modulation as a function of the angle of incidence or wavelength of the incident light; and

determining the angle of incidence or wavelength at which the intensity of the light reflected from the periodic refractive index modulation is a maximum.

2. A method as claimed in Claim 1, wherein the arrangement is such that at the angle of incidence or wavelength at which the intensity of the light reflected is a maximum there is destructive interference of transmitted components of the incident light.

3. A method as claimed in Claim 1 or Claim 2, wherein the arrangement is such that the

radius of the incident beam at the grating exceeds the coupling length of the guided mode in the waveguide, and the dissipative and scattering losses in the waveguide are far less than the radiative losses.

4. A method as claimed in any preceding claim, wherein the maximum intensity of the reflected light is greater than 20% of the intensity of the light incident on the substrate.

5. A method as claimed in claim 4, wherein the maximum intensity of the reflected light is greater than 30% of the intensity of the light incident on the substrate.

6. A method as claimed in any preceding claim, wherein the periodic refractive index modulation is a surface relief profile or a grating formed in the surface of the substrate to which the waveguide is applied and/or in the surface of the waveguide on which the second molecular species is immobilized.

7. A method as claimed in Claim 6, wherein the surface relief profile is sinusoidal, rectangular, triangular or trapezoidal.

8. A method as claimed in any preceding claim, wherein the substrate is a chip of glass or silica.

9. A method as claimed in any preceding claim, wherein the waveguide has a refractive index of between 1.80 and 2.50.

10. A method as claimed in any preceding claim, wherein the waveguide is of a material selected from the group consisting of hafnium dioxide, silicon nitride, tantalum pentoxide and titanium oxide.

11. A method as claimed in any preceding claim, wherein the waveguide has a thickness of 100nm to 200nm.

12. A method as claimed in any preceding claim, wherein the waveguide has a thickness of 140nm to 180nm.

13. A method as claimed in any preceding claim, wherein the depth of the periodic refractive index modulation is less than 50nm.

14. A method as claimed in any preceding claim, wherein the depth of the periodic refractive index modulation is between 2nm and 20nm.

15. A method as claimed in any preceding claim, wherein the period of the periodic refractive index modulation is between 600nm and 1000nm.

16. A method as claimed in any preceding claim, wherein the beam of incident monochromatic light is TM-polarized.

17. A method as claimed in any preceding claim, wherein the angle of incidence of the beam of incident monochromatic light is, or is close to, the Brewster angle, or the range of angles of incidence includes the Brewster angle.

18. A method as claimed in any preceding claim, wherein the beam of incident light has a diameter of less than 10mm, eg 1 to 8mm.

19. A method as claimed in any preceding claim, wherein the point at which the beam of incident light is incident upon the periodic refractive index modulation is offset from the centre of that modulation.

20. A method as claimed in Claim 19, wherein the length of the periodic refractive index modulation is less than 10mm and the incident light beam is offset from the centre of the modulation by less than 1mm.

21. A method as claimed in any preceding claim, wherein the beam of incident light has a

fixed wavelength λ, and the waveguide thickness is in the range $\lambda/12$ to $\lambda/2$, the depth of the periodic refractive index modulation is in the range $\lambda/300$ to $\lambda/12$, and the period of the modulation is in the range $\lambda/2$ to 2λ.

22. A method as claimed in any preceding claim, wherein the angle of incidence or wavelength at which the intensity of the reflected light is a maximum is determined before and after the first and second molecular species are brought into contact.

23. A method as claimed in any one of Claims 1 to 21, wherein the angle of incidence or wavelength at which the intensity of the reflected light is a maximum is determined periodically or continuously in real time after the first and second molecular species are brought into contact.

24. Apparatus for monitoring the interaction of a first molecular species with a second molecular species comprises

a sensor device comprising a substrate having a waveguide formed on at least part of the surface thereof, the waveguide having a first major surface which constitutes an interface between the waveguide and the substrate and a second major surface upon which the second molecular species is immobilized, at least a region of the first and/or second major surface being formed with a periodic refractive index modulation;

means for irradiating said periodic refractive index modulation with a beam of incident monochromatic light,

means for varying the angle of incidence of said light or the wavelength of said light through a range of angles or wavelengths respectively, said range including an angle or a wavelength, as the case may be, at which a guided mode is excited in said waveguide; and

means for monitoring the intensity of light reflected from said periodic refractive index modulation as a function of the angle of incidence or wavelength of the incident light;

means for determining the angle of incidence or wavelength at which the intensity of the reflected light is a maximum.

25. Apparatus as claimed in Claim 24, wherein the arrangement is such that at the angle of incidence or wavelength at which the intensity of the light reflected is a maximum there is destructive interference of transmitted components of the incident light.

26. Apparatus as claimed in Claim 24 or Claim 25, wherein the arrangement is such that the radius of the incident beam at the grating exceeds the coupling length of the guided mode in the waveguide, and the dissipative and scattering losses in the waveguide are far less than the radiative losses.

27. Apparatus as claimed in claim 26, wherein the maximum intensity of the reflected light is greater than 20% of the intensity of the light incident on the substrate.

28. Apparatus as claimed in claim 27, wherein the maximum intensity of the reflected light is greater than 30% of the intensity of the light incident on the substrate.

29. Apparatus as claimed in any one or Claims 24 to 28, wherein the periodic refractive index modulation is a surface relief profile or a grating formed in the surface of the substrate to which the waveguide is applied and/or in the surface of the waveguide on which the second molecular species is immobilized.

30. Apparatus as claimed in Claim 29, wherein the surface relief profile is sinusoidal, rectangular, triangular or trapezoidal.

31. Apparatus as claimed in any one of Claims 26 to 30, wherein the substrate is a chip of glass or silica.

32. Apparatus as claimed in any one of Claims 26 to 31, wherein the waveguide has a refractive index of between 1.80 and 2.50.

33. Apparatus as claimed in any one of Claims 26 to 32, wherein the waveguide is of a material selected from the group consisting of hafnium dioxide, silicon nitride, tantalum pentoxide and titanium oxide.

34. Apparatus as claimed in any one of Claims 26 to 33, wherein the waveguide has a thickness of 100nm to 200nm.

35. Apparatus as claimed in any one of Claims 26 to 34, wherein the waveguide has a thickness of 140nm to 180nm.

36. Apparatus as claimed in any one of Claims 26 to 35, wherein the depth of the periodic refractive index modulation is less than 50nm.

37. Apparatus as claimed in any one of Claims 26 to 36, wherein the depth of the periodic refractive index modulation is between 2nm and 20nm.

38. Apparatus as claimed in any one of Claims 26 to 37, wherein the period of the periodic refractive index modulation is between 600nm and 1000nm.

39. Apparatus as claimed in any one of Claims 26 to 38, wherein the beam of incident light has a diameter of less than 10mm, eg 1 to 8mm.

40. Apparatus as claimed in any one of Claims 26 to 39, wherein the point at which the beam of incident light is incident upon the periodic refractive index modulation be offset from the centre of that modulation.

41. Apparatus as claimed in Claim 40, wherein the length of the periodic refractive index modulation is less than 10mm and the incident light beam is offset from the centre of the modulation by less than 1mm.

42. Apparatus as claimed in any one of Claims 26 to 41, wherein the beam of incident light

has a fixed wavelength λ, and the waveguide thickness is in the range $\lambda/12$ to $\lambda/2$, the depth of the periodic refractive index modulation is in the range $\lambda/300$ to $\lambda/12$, and the period of the modulation is in the range $\lambda/2$ to 2λ.

43. Apparatus as claimed in any one of Claims 26 to 42, further comprising means for polarizing the incident light beam such that the light incident upon the sensor device is TM-polarized.

44. Apparatus as claimed in Claim 43, wherein the angle of incidence of the beam of incident monochromatic light is, or is close to, the Brewster angle, or the range of angles of incidence includes the Brewster angle.

45. A method of fabricating a sensor device for use in the method of any one of claims 1 to 25, which comprises

a) coating a substrate with a masking material;
b) removing parts of the masking material to reveal exposed substrate regions;
c) etching the surface of the substrate in the exposed substrate regions;
d) removing all remaining masking material; and
e) depositing a waveguide on the surface of the substrate.

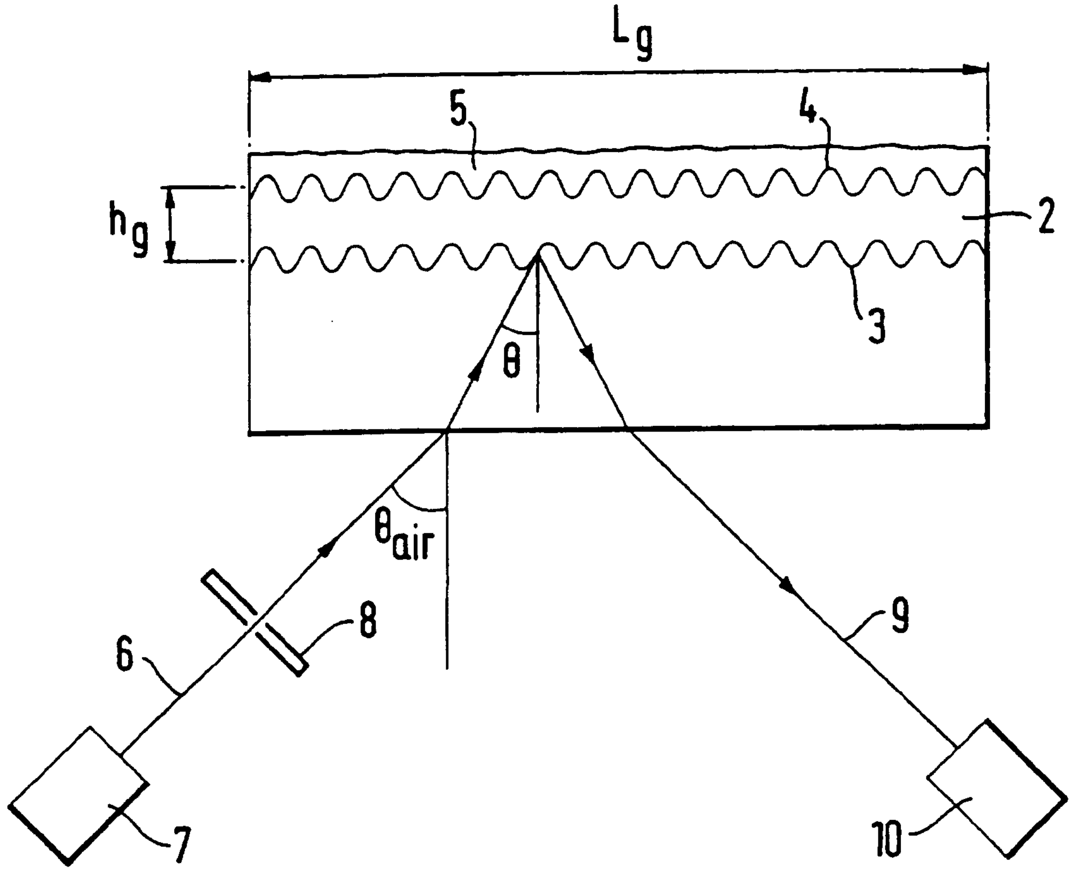

Fig.1

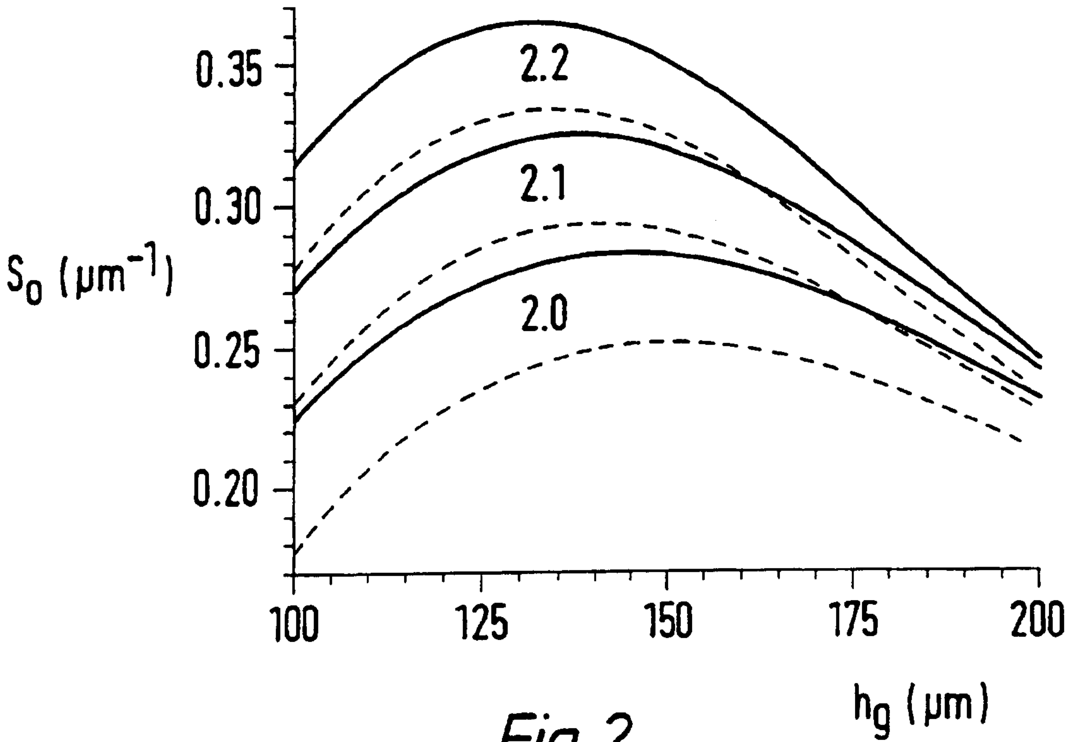
0.35
0.30
0.25
0.20
S_0 (μm^{-1})
2.2
2.1
2.0
100
125
150
175
200
h_g (μm)

Fig.2

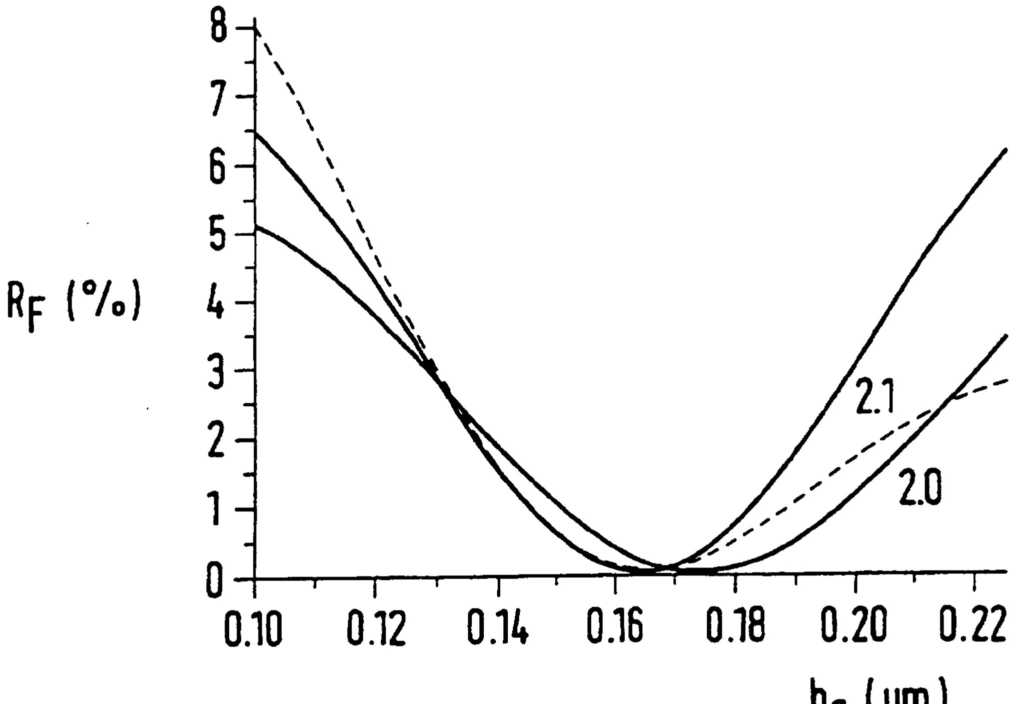
8
7
6
5
4
3
2
1
0
R_F (%)
2.1
2.0
0.10
0.12
0.14
0.16
0.18
0.20
0.22
h_g (μm)

Fig.3

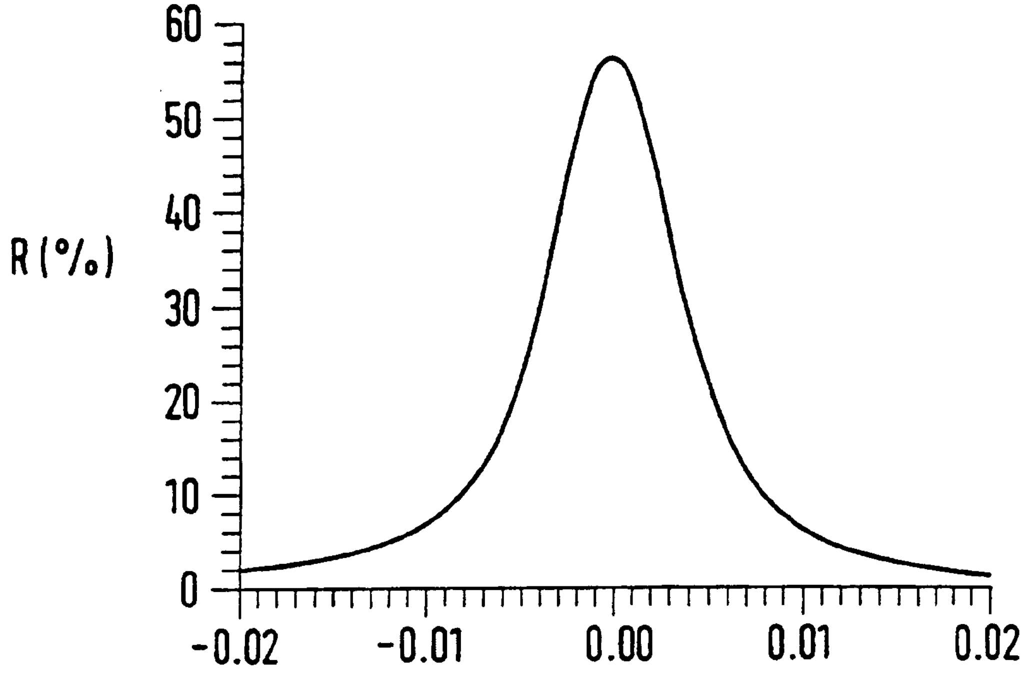
60
50
40
R (%)
30
20
10
0
-0.02
-0.01
0.00
0.01
0.02
θ (°)

Fig. 4

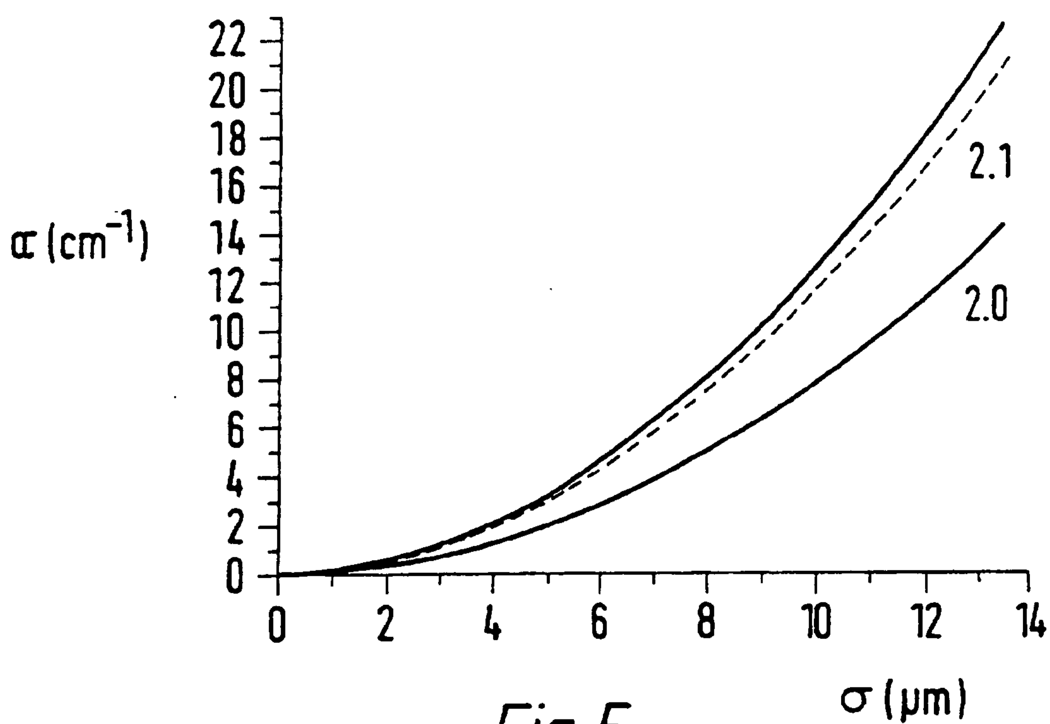
22
20
18
16
α (cm⁻¹)
14
12
10
8
6
4
2
0
2.1
2.0
0
2
4
6
8
10
12
14
σ (μm)

Fig. 5

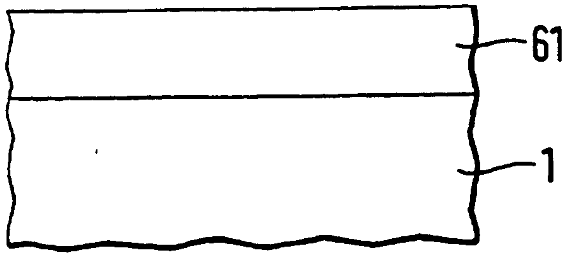

Fig.6a

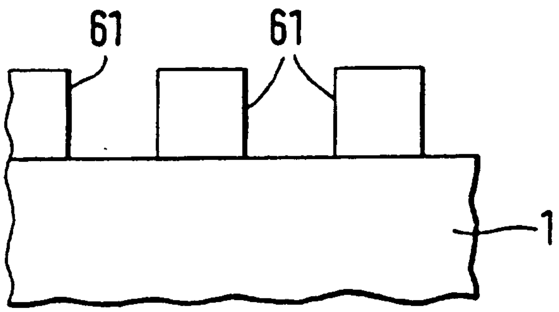

Fig.6b

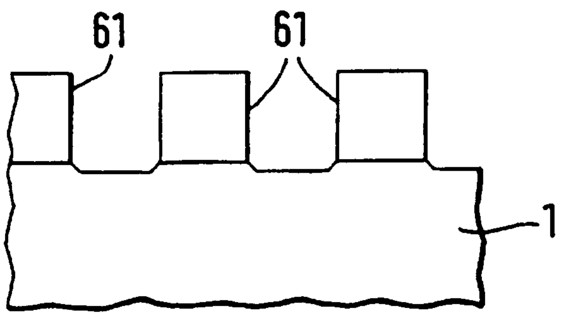

Fig.6c

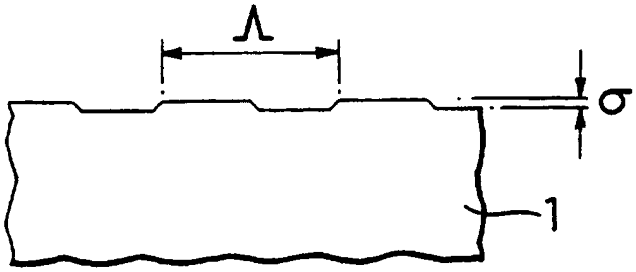

Fig.6d

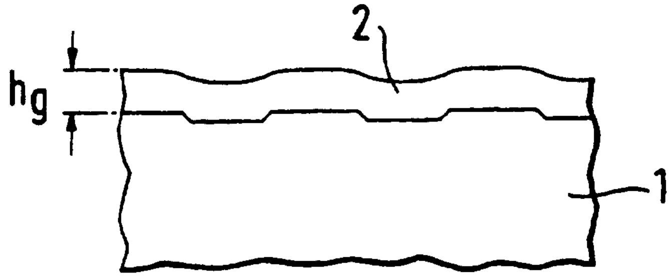

Fig.6e

Intern. ...al Application No
PCT/GB 97/00346

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N21/77

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 455 178 A (FATTINGER) 3 October 1995	1,6-8, 10,19, 24, 29-31, 33,40
	see column 1, line 10 - line 16 see column 3, line 5 - line 19 see column 4, line 39 - line 55 see column 5, line 58 - line 62 see column 5, line 62 - column 6, line 6 see column 6, last paragraph - column 7, line 26	
A	see column 9, line 16 - line 39; figure 4	3,18,20, 26,39, 41,45

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
24 April 1997	07.05.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Thomas, R.M.

Inter nal Application No
PCT/GB 97/00346

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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